

Genetic characterization of European HPAI H5Nx viruses identified in Bulgaria, Germany and Hungary between 11th February and 22nd March 2020

Present situation

The following sequences have been produced by the EURL, deposited in GISAID or provided by Member States:

Virus	Virus subtype	Country	Collection Date	Available sequences
A/chicken/Germany-SN/AI00276/2020	H5N8	Germany	12/03/2020	Whole genome
A/buzzard/Germany-SN/AI00285/2020	H5N8	Germany	21/03/2020	Whole genome
A/turkey/Germany-NI/AI00334/2020	H5N8	Germany	21/03/2020	Whole genome
A/laying hen/Poland/312/2020	H5N8	Poland	06/03/2020	Partial HA and NA
A/duck/Hungary/14969/2020/H5N8	H5N8	Hungary	22/03/2020	Partial HA
A/mule duck/Bulgaria/50-506_20VIR1414-1/2020 <i>Intestine (1st shipment). Outbreak 1</i>	H5N2/H5N8	Bulgaria	11/02/2020	Partial genome
A/duck/Bulgaria/78-4t_20VIR1416-3/2020 <i>Swab (1st shipment). Outbreak 2</i>	H5N8	Bulgaria	21/02/2020	Partial genome
A/chicken/Bulgaria/77-23_20VIR1415-1/2020 <i>Intestine (1st shipment). Outbreak 3</i>	H5N2	Bulgaria	21/02/2020	Partial genome
A/mule duck/Bulgaria/148_20VIR1722-1/2020 <i>Swab (2nd shipment). Outbreak 4</i>	NEG	Bulgaria	21/02/2020	NA
A/mule duck/Bulgaria/147_20VIR1721-1/2020 <i>Swab (2nd shipment). Outbreak 5</i>	H5N8	Bulgaria	21/02/2020	Partial genome
A/chicken/Bulgaria/201_20VIR1723-1/2020 <i>Swab (2nd shipment). Outbreak 6</i>	H5N2	Bulgaria	02/03/2020	Partial genome
A/chicken/Bulgaria/217_20VIR1724-1/2020 <i>Swab (2nd shipment). Outbreak 7</i>	H5N8	Bulgaria	09/03/2020	Partial genome
A/chicken/Bulgaria/221_20VIR1725-1/2020 <i>Swab (2nd shipment). Outbreak 8</i>	H5N2	Bulgaria	11/03/2020	Partial genome
A/mule duck/Bulgaria/50_20VIR1726/2020 <i>Isolate (Bulgaria) from outbreak 1 (2nd shipment)</i>	H5N2/H5N8	Bulgaria	11/02/2020	Whole genome
A/chicken/Bulgaria/77_20VIR1727/2020 <i>Isolate (Bulgaria) from outbreak 3 (2nd shipment)</i>	H5N2	Bulgaria	21/02/2020	Whole genome

H5N8/H5N2 co-infection was confirmed in the isolate of the first outbreak recently sent by the Bulgarian colleagues.

Samples sent to EURL from Bulgarian outbreak 2020/4 (id. 148) resulted negative.

Phylogenetic analysis

Overview

The HA gene of all the analysed HPAI viruses belongs to clade 2.3.4.4b. Sequences available from the recent H5N8 viruses from Germany, Hungary and Poland cluster with the H5N8 viruses circulating in East-central Europe since December 2019.

We confirmed the co-circulation of two HPAI subtypes in Bulgaria: H5N8 and H5N2. The H5N8 viruses have the same gene composition of the H5N8 circulating in Bulgaria in 2018-2019, while the H5N2 is a reassortant strain, with the HA and M genes related to the Bulgarian H5N8 viruses and the other genes correlated with LPAI viruses collected in Eurasia from domestic and wild birds.

Beside the outbreak 2020/1 in mule ducks, which resulted co-infected with both H5N8 and H5N2 subtypes, the H5N2 viruses were all collected from layer hens farms, while the H5N8 viruses were all identified in ducks farms.

Find below a detailed description of the viruses identified in the different countries.

H5N8 from Germany

Complete genome sequences of the three H5N8 viruses identified in a backyard poultry holding, in a turkey breeder farm and in a common buzzard in Germany cluster together and with the virus responsible of the first H5N8 outbreak (February 2020) in the country. Specifically the two viruses collected from the backyard poultry holding (A/chicken/Germany-SN/AI00276/2020) in Leipzig and from the wild bird case (A/buzzard/Germany-SN/AI00285/2020), which was reported in the same area, were closely related (Figures 1 to 8).

H5N8 from Poland

The partial HA and NA sequences of the H5N8 virus collected from the last outbreak in a backyard holding in Poland (6th March 2020) cluster with the H5N8 viruses recently identified in Germany.

H5N8 from Hungary

The partial HA sequence of the H5N8 virus responsible of one of the recent outbreaks in ducks in Hungary clusters with all the other European H5N8 viruses. In particular, it shows the highest identity (99.8%) with H5N8 viruses from Romania and Slovakia.

H5N8 and H5N2 from Bulgaria

The HPAI viruses collected in Bulgaria belong to the H5N8 (outbreaks 2020/2, 5 and 7) and H5N2 (outbreaks 2020/3, 6 and 8) subtypes, indicating the co-circulation in the country of two distinct strains. The isolate from the first outbreak (2020/1), recently sent from the Bulgarian colleagues, was confirmed to be co-infected with H5N8 and H5N2 viral subtypes. For this outbreak, sequences of the complete genome of the H5N2 subtype and the N8 gene of the H5N8 subtype were obtained.



The eight gene segments of the H5N8 viruses cluster together and with H5N8 viruses circulating in Bulgaria in 2018-19. In particular, H5N8 viruses from outbreaks 2020/2 (ducks) and 5 (Mule ducks) collected from Plovdiv are highly related (similarity of 99.7% for the HA gene), while virus from outbreak 2020/7 (layer hens) collected from Kurdzhali shows an identity of 99.2-99.4% for the HA gene with the other two viruses (Figures 1 to 8).

Outbreaks 2020/1 (mule ducks, co-infected with a H5N8 virus), 3 (hens), 6 (hens) and 8 (hens) were confirmed to be caused by a H5N2 reassortant strain. Specifically, the HA and M genes (Figures 1 and 7) cluster with H5N8 viruses circulating in Bulgaria in 2018-2020; the N2 gene clusters with H6N2 viruses identified in mule ducks in Bulgaria in 2009-2010 (Figure 9); the other gene segments cluster with LPAI viruses identified in wild and domestic birds in Eurasia. Of note, the NS gene segment of the Bulgarian H5N2 viruses belongs to allele B, while the H5N8 viruses possess the allele A (Figure 8).

Bulgarian HPAI H5N2 differs from Taiwanese HPAI H5N2, which caused 31 outbreaks in chickens, ducks, geese and turkeys between 16 November 2019 and 15 February 2020.

Molecular analysis

To date, there is no evidence of mutations associated with mammalian adaptation in all the analysed viruses.

The H5N2 viruses obtained from outbreak 1 and 3 possess a potential additional glycosylation site in position 70-72 (D70N) of the HA gene (numbering starting from the first methionine) which indicates an adaptation of the virus to domestic birds.

Potential additional glycosylation sites were identified in the NA protein of the H5N8 virus A/chicken/Bulgaria/217_20VIR1724-1/2020 (outbreak 2020/7) in position 411-413 (ELS to NLS) and A/chicken/Germany-SN/AI00276/2020 in position 263-265 (DQT to NQT).

HA

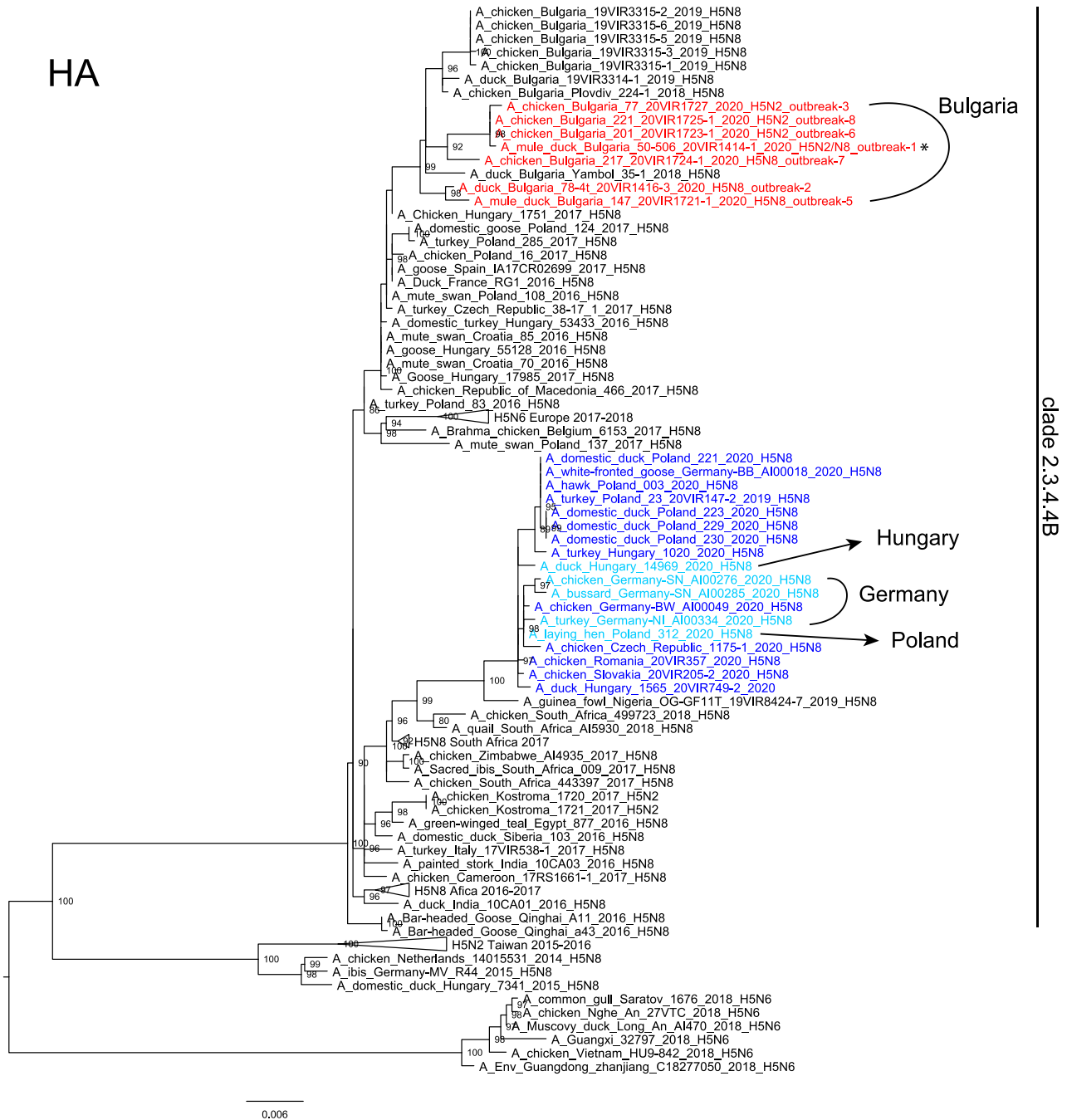


Fig.1. Maximum Likelihood phylogenetic tree of the **HA gene** (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red, the H5N8 viruses currently circulating in East-Central Europe are marked in blue (recent viruses in light blue). Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. *identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N2 virus.

NA - N8 subtype



Fig.2. Maximum Likelihood phylogenetic tree of the **N8 gene** (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red, the H5N8 viruses currently circulating in East-Central Europe are marked in blue (recent viruses in light blue). Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. *identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N8 virus.

PB2



Fig.3. Maximum Likelihood phylogenetic tree of the **PB2 gene** (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red, the H5N8 viruses currently circulating in East-Central Europe are marked in blue (recent viruses in light blue). Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. *identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N2 virus.

PB1



Fig.4. Maximum Likelihood phylogenetic tree of the **PB1 gene** (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red, the H5N8 viruses currently circulating in East-Central Europe are marked in blue (recent viruses in light blue). Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. * identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N2 virus.

PA

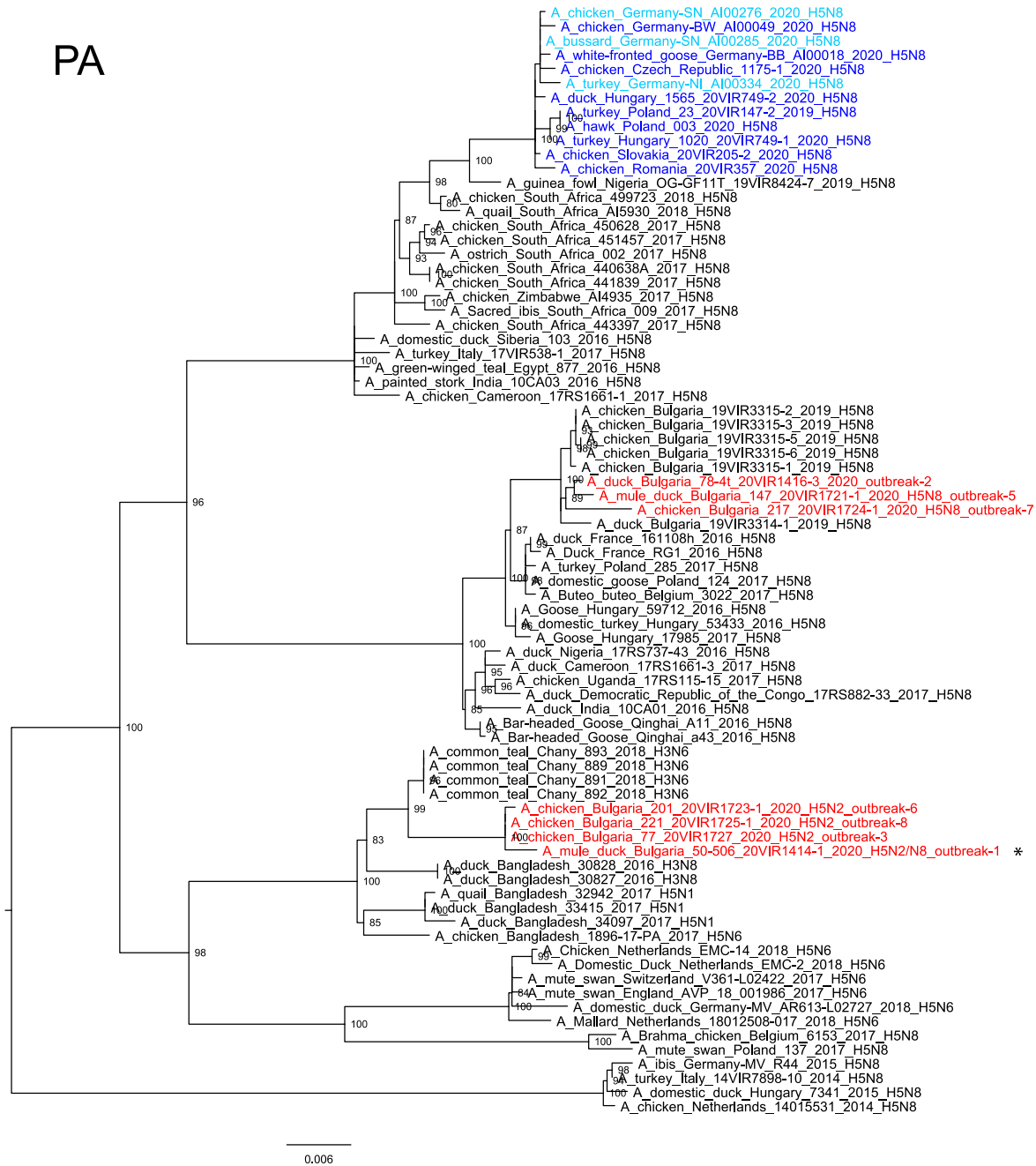


Fig.5. Maximum Likelihood phylogenetic tree of the **PA gene** (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red, the H5N8 viruses currently circulating in East-Central Europe are marked in blue (recent viruses in light blue). Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. * identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N2 virus.

NP

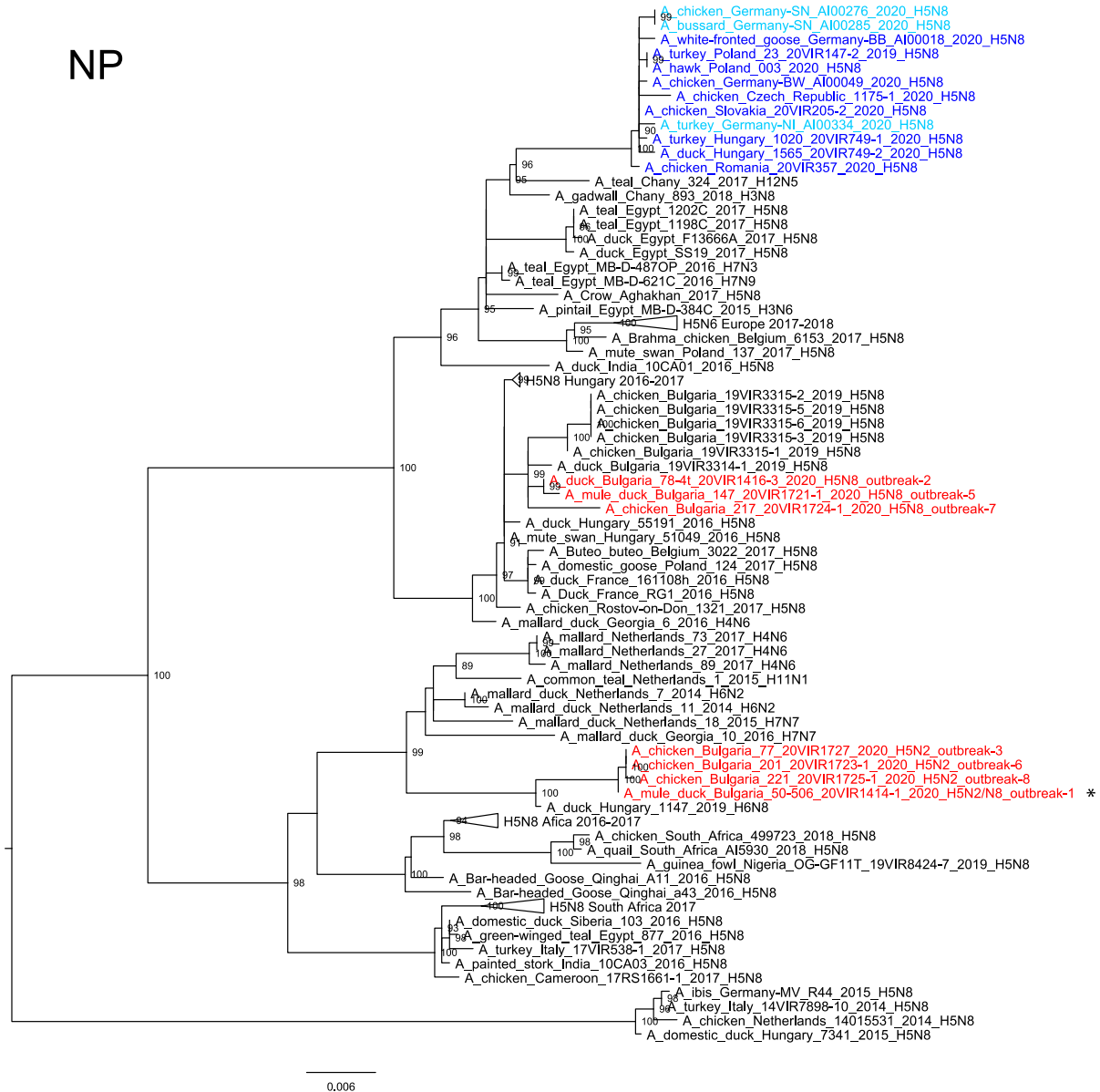


Fig.6. Maximum Likelihood phylogenetic tree of the **NP gene** (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red, the H5N8 viruses currently circulating in East-Central Europe are marked in blue (recent viruses in light blue). Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. *identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N2 virus.

M

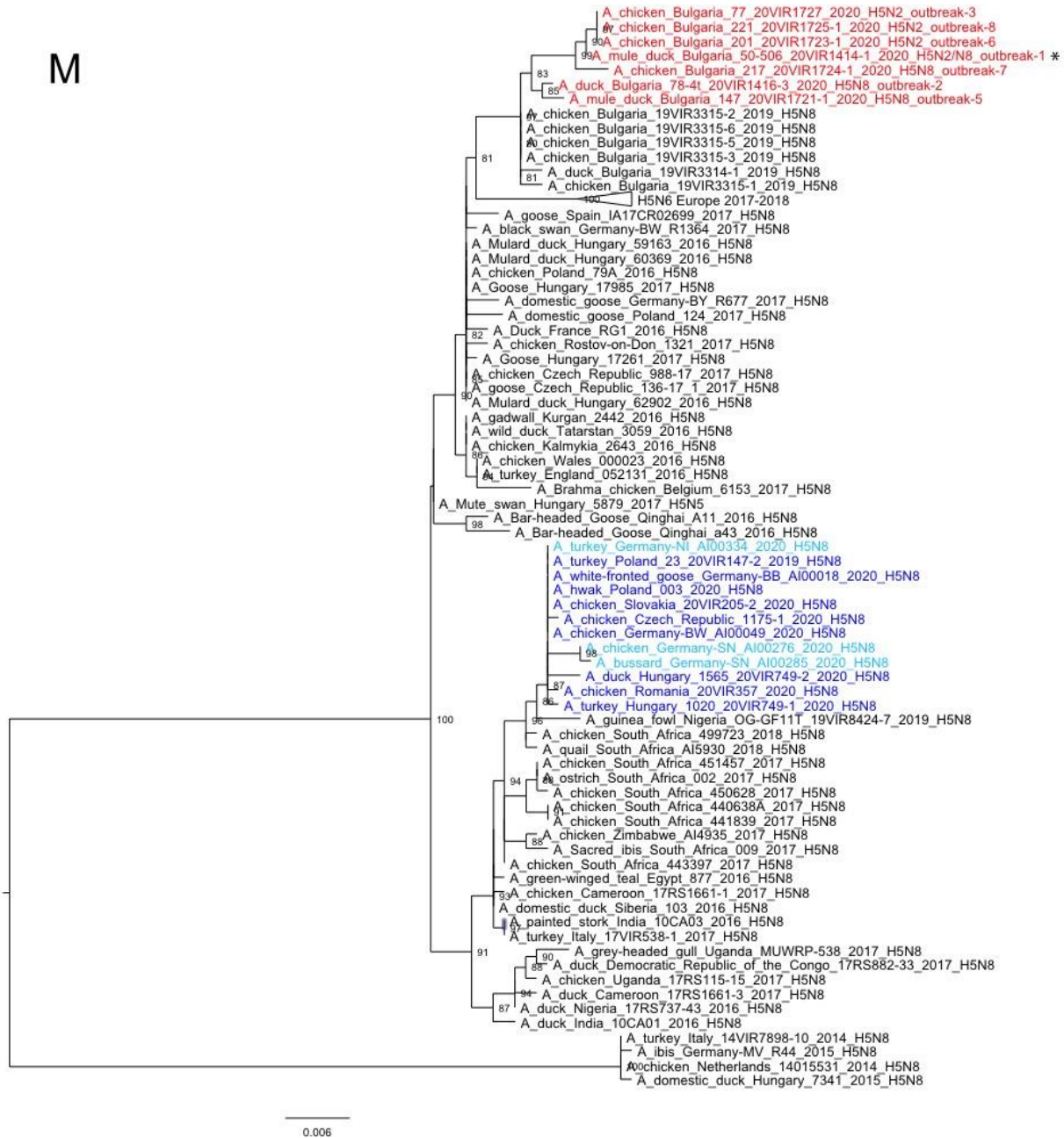


Fig.7. Maximum Likelihood phylogenetic tree of the **M** gene (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red, the H5N8 viruses currently circulating in East-Central Europe are marked in blue (recent viruses in light blue). Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. *identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N2 virus.

NS



Fig.8. Maximum Likelihood phylogenetic tree of the NS gene (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red, the H5N8 viruses currently circulating in East-Central Europe are marked in blue (recent viruses in light blue). Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. *identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N2 virus.

NA - N2 subtype

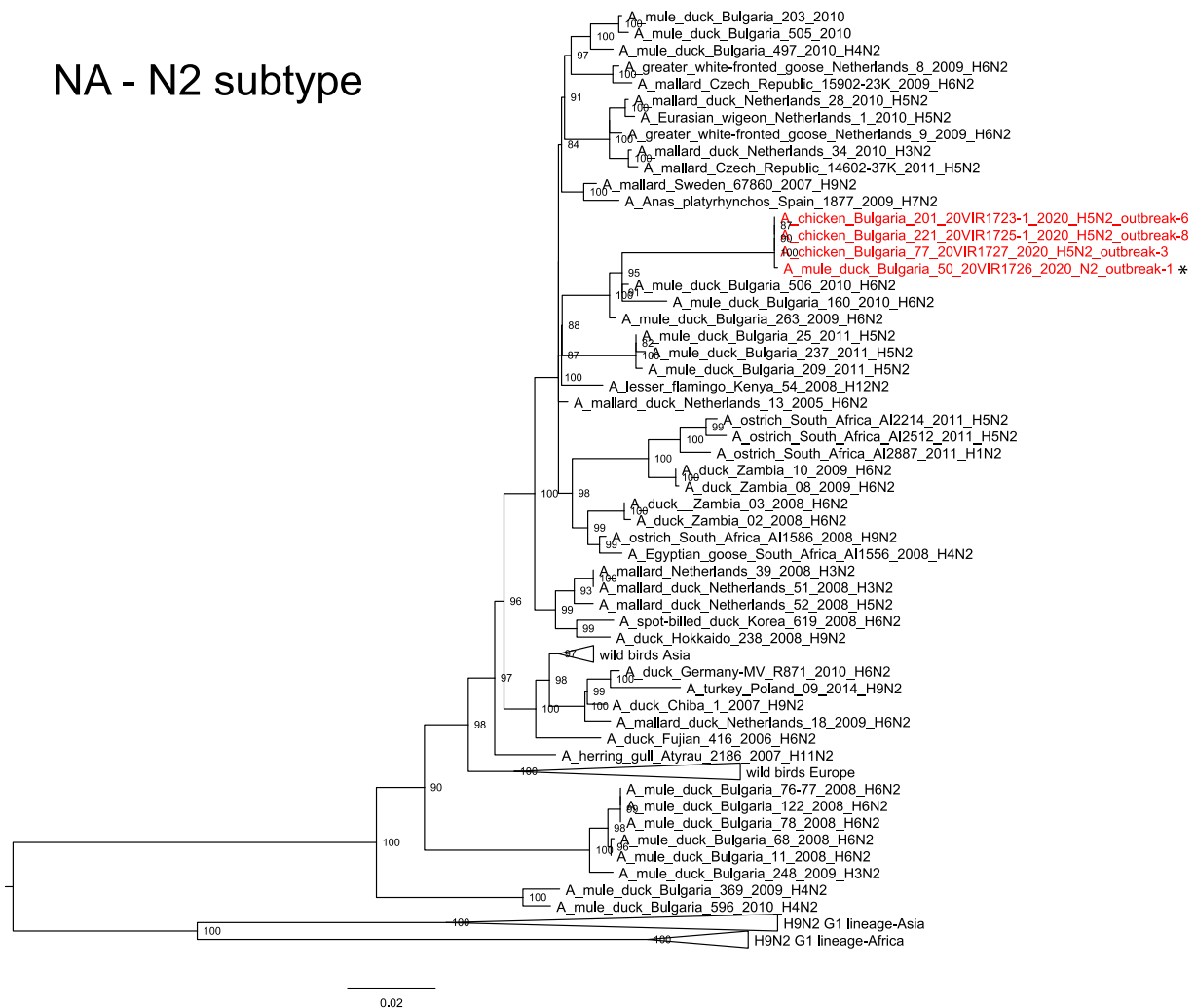


Fig.9. Maximum Likelihood phylogenetic tree of the **N2 gene** (IQ-TREE v.1.6.8). The new Bulgarian sequences are marked in red. Ultra-fast bootstrap supports higher than 80% are indicated next to the nodes. *identifies the H5N2/N8 co-infected outbreak 1. The sequence showed here corresponds to the H5N2 virus.